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Marcos F.T. Oliveira  
*Gulf Coast Research Laboratory*

William E. Hawkins  
*Gulf Coast Research Laboratory, William.Hawkins@usm.edu*

Robin M. Overstreet  
*Gulf Coast Research Laboratory, robin.overstreet@usm.edu*

William W. Walker  
*Gulf Coast Research Laboratory*

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## HEPATOCELLULAR NEOPLASM IN A WILD-CAUGHT SHEEPSHEAD MINNOW (*CYPRINODON VARIEGATUS*) FROM THE NORTHERN GULF OF MEXICO

MARCOS F.T. OLIVEIRA, WILLIAM E. HAWKINS,  
ROBIN M. OVERSTREET AND WILLIAM W. WALKER  
Gulf Coast Research Laboratory, P.O. Box 7000, Ocean Springs, Mississippi  
39566-7000

### INTRODUCTION

The sheepshead minnow, *Cyprinodon variegatus*, is a widely distributed small fish species that inhabits estuaries and inshore waters from New England to northern South America. This species has been used extensively in aquatic environmental toxicity and carcinogenicity tests (Couch *et al.*, 1981; Courtney and Couch, 1984). Hepatic neoplasms have been induced in the sheepshead minnow by exposure to several known chemical carcinogens including diethylnitrosamine (Couch and Courtney, 1987), methylazoxymethanol acetate (Hawkins *et al.*, 1985), and dimethylbenz(a)anthracene (Hawkins *et al.*, 1991). Because of its widespread distribution, limited home range, and proven sensitivity to carcinogens, the sheepshead minnow appears to be a good candidate to serve as an *in situ* monitor of environmental carcinogens and other toxicants in coastal waters, especially those of the Gulf of Mexico and Caribbean Sea. To establish background information on the histopathological lesions in sheepshead minnows taken from the wild, we collected and examined specimens from an offshore site presumed to be free of chemical contamination. The present report concerns a hepatic neoplastic lesion, diagnosed as a hepatocellular adenoma, found in a wild sheepshead minnow. Neoplasms from wild sheepshead minnows or spontaneous neoplasms from laboratory specimens previously have not been reported.

### MATERIALS AND METHODS

The collecting site was Big Lagoon on Horn Island in the Mississippi Sound approximately 18 kilometers from the mainland. Horn Island is part of the Gulf Islands National Seashore of the United States Park Service. About 300 specimens were collected by seining, returned to the laboratory under aeration, anesthetized with tricaine methanesulfonate (TMS), and examined for external lesions. After internal organs were examined for visible

lesions, the liver, gills, and kidney were removed, fixed in Lillie's solution (Humason, 1972) for 48 hours, washed in running water for 24 hours, stored in 70% ethanol at room temperature, cleared in Shandon xylene substitute (Shandon Inc., Pittsburg, PA) and embedded in paraffin following routine procedures. Sections were cut on a rotary microtome at 5 to 6  $\mu$ m thickness and stained with hematoxylin and eosin.

### RESULTS AND DISCUSSION

A neoplastic lesion was found in the liver of a sheepshead minnow collected from Big Lagoon on Horn Island. No other signs of disease were observed. The specimen was a female estimated to be about one year old. In the plane of section in which it was detected, the neoplasm was 1.1 mm by 0.78 mm and occupied approximately 12% of the liver (Figure 1).

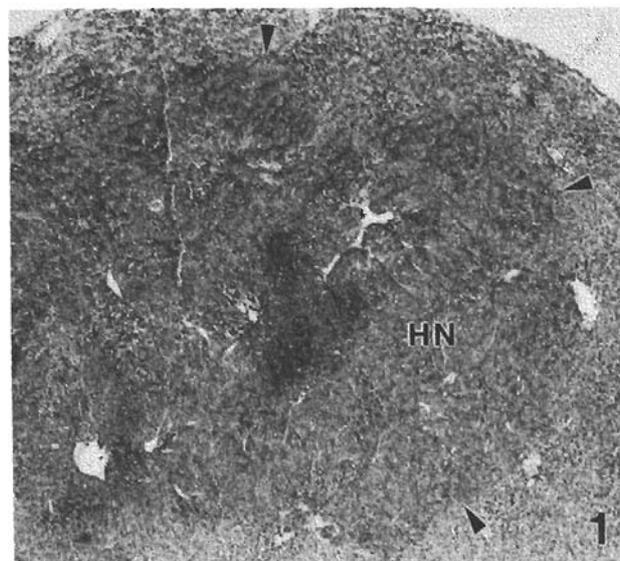


Figure 1. Low power micrograph showing hepatocellular neoplasm (HN) occupying a large portion of the liver of a sheepshead minnow. Arrowheads indicate border of lesion with surrounding normal tissue. Hematoxylin and eosin.

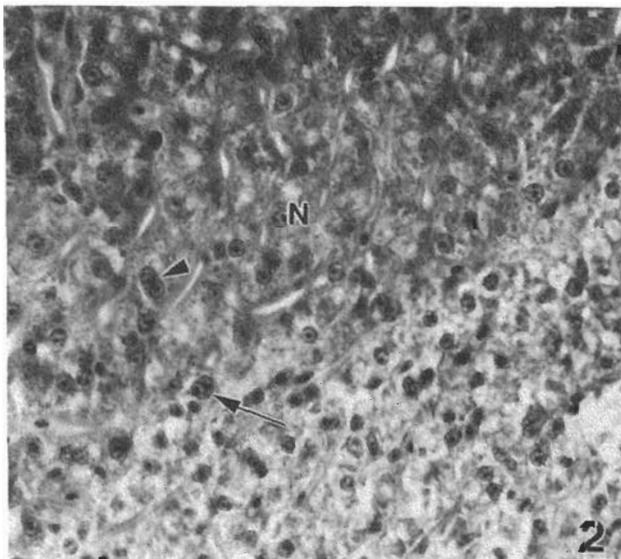
We diagnosed the lesion as a hepatocellular adenoma, but recognize that depending on the pathologist's particular criteria, a diagnosis of hepatocellular carcinoma might be justified. In our opinion, however, most of the criteria fit the diagnosis of adenoma. Cytologically, the lesion was well differentiated. That is, the organization and form of the cells in the lesion resembled those features in surrounding normal tissues (Figure 2). The neoplastic hepatocytes were larger and had larger nuclei than normal hepatocytes. Several mitotic figures were observed in the lesion (Figure 3). At its periphery, the lesion appeared to compress adjacent normal liver. No other neoplastic lesions were found in sections from specimens from that collection site.

Epizootics of hepatic neoplasms in fishes are highly correlated with environmental contamination (Harshbarger and Clark, 1990; Harshbarger *et al.*, 1993). Those authors considered an epizootic to be the occurrence of three or more cases of a neoplastic lesion from a specific cell lineage occurring in a single species from a defined geographic location. Although most neoplasm epizootics have involved large fish species, Vogelbein *et al.* (1990) recently reported epizootic hepatic neoplasia in a large percentage of mummichog (*Fundulus heteroclitus*) from a creosote-contaminated site along the Elizabeth River, VA. This present case of a hepatocellular neoplasm in a sheepshead minnow does not constitute an epizootic. The specimen

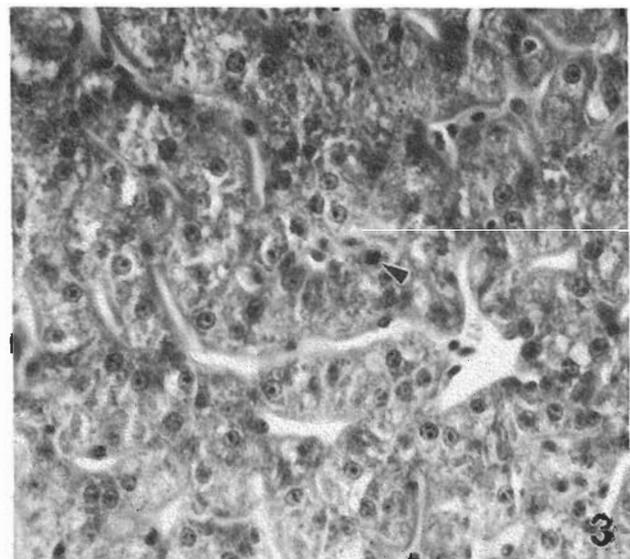
was collected from a site considered to be uncontaminated by chemicals. The lesion is significant, however, because it is the first report of a hepatic neoplasm in a wild sheepshead minnow and, possibly, is the first report of a hepatic neoplasm from a fish from the Gulf of Mexico. The occurrence of a single neoplasm-bearing specimen from over 300 examined fish could represent a spontaneous or background rate. Couch and Courtney (1987), however, reported no spontaneous liver neoplasms in several thousand sheepshead minnows examined over about 15 years. Nevertheless, the sensitivity of the sheepshead minnow to several carcinogens in laboratory and the widespread geographic distribution of the species suggest that the sheepshead minnow would be a good subject for surveys of aquatic environmental carcinogens in warm southern marine and estuarine waters.

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**Figure 2.** Higher power micrograph showing boundary between neoplastic cells (N) and normal surrounding tissue. Note binucleate cell (arrowhead) and enlarged nucleus (arrow) in lesion. Hematoxylin and eosin.



**Figure 3.** Mitotic figure (arrowhead) in lesion. Hematoxylin and eosin.

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